Adenoviral Reference Material Working Group
Canji & SPRI Group RFP 10 Bid Submission Form
Participation in Other Characterization of Reference Material –

Please complete the following fields: This is a group submission. Beth Hutchins is the group coordinator.

Contact Information – RFP 10.0

*Contact Individual:	Dr. Beth Hutchins
Institution:	Canji, Inc.
Address:	3525 John Hopkins Ct. San Diego, CA 92121
Phone Number:	858-646-5930
Fax Number:	858-597-0237
Email Address:	beth.hutchins@canji.com

***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- [X] Determination of Particle Concentration
- [ ] Determination of Infectious Titer
- [ ] Short-term/Field Stability Studies
- [ ] Long-term Stability Study
- [ ] Donation of Supplies/Other Services for Characterization Phase

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*Contact Individual:	Dr. Gary Vellekamp
Institution:	Schering Plough Research Institute
Address:	1011 Morris Ave., U-13-1 Union, NJ 07083
Phone Number:	908-820-6181
Fax Number:	908-820-6785
Email Address:	gary.vellekamp@spcorp.com

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Joint Canji/SPRI Submission

Canji and SPRI propose to perform additional characterization of the Adenovirus 5 WT Reference Material:

- Free Hexon Determination – a measure of purity, Free hexon contaminants may result from either co-purification of a small fraction of the unassembled hexons, or hexon dissociation from the virus due to limited instability of the virus capsid. The immunoaffinity/gel filtration assay uses a commercially-available fluorescently-labeled antibody that binds to free hexon but not to hexon of the virus capsid (to be performed by SPRI).
- 31K MW Protein Level – quantifies the amount of 31K protein (pVIII) by RP-HPLC. This protein may indicate the amount of a particular adenoviral precursor form in the preparation; it may be indicative of empty capsid contamination (to be performed by SPRI).
- Residual 293 Host Cell Protein – a measure of purity assessed by ELISA and by Western blot using commercially available reagents and kits from Cygnus Technologies (Plainville, MA) (to be performed by Canji).

Canji, Inc. is a wholly owned subsidiary of Schering Plough Corp., specializing in adenoviral vector gene therapy including pharmacology, analytical methods development, and process development. Canji's Process Sciences' personnel have a well-established reputation for expertise in adenoviral vector technology, evidenced by publications and issued patents related to production using HEK-293 cells and characterization methods. The Residual 293 Host Cell Protein analyses would be performed under the guidance of Dr. Barry Sugarman.

SPRI is the R&D product development subsidiary of Schering Plough Corp. All work there would be performed under the guidance of Dr. Gary Vellekamp in his research laboratory. Dr. Vellekamp has worked on adenoviral vectors since 1995 when SP purchased the rAd-p53 project from Canji.

Both Canji and SPRI are able to begin these analyses within 3 weeks of receipt of the Ad5 WT Reference Material vials. Data in the form of reports would be submitted to the WG within 4 weeks of the initiation of analyses.

The Free Hexon Determination method and 31K MW Protein Level test require 1 vial of the Reference Material altogether. The Residual 293 Host Cell Protein analyses require 1 vial of the Reference Material.

To support this bid, a total of 2 vials of the Reference Material are required.

Method Descriptions

Free Hexon Determination - Hexon content is assessed using an immunoaffinity/gel filtration assay. Sample is processed chromatographically and eluted fractions are assessed quantitatively for binding to a commercially-available fluorescently-labeled anti-hexon antibody. The labeled anti-hexon antibody binds to free hexon but not to hexon of the virus capsid, allowing quantitation against a hexon standard curve.

31K MW Protein Level – An RP-HPLC assay quantifies the amount of 31K protein (pVIII). The RP-HPLC assay described by Lehmberg, E., Traina, J.A., Chakel, J.A., Chang, R.-J., Parkman, M., McCaman, M.T., Murakami, P.K., Lahidji, V., Nelson, J.W., Hancock, W.S., Nestaas, E., and E. Pungor, Jr. (1999, "Reversed-phase high performance liquid chromatographic assay for the adenovirus type 5 proteome," *J. Chromatogr. B. 732,* 411—423) is performed as described, except that the column temperature is 50ºC and the 20% to 40% segment of the gradient is extended to 10 minutes. A secondary method for determination of 31K MW protein content is a SDS-PAGE method. 31K MW protein content is estimated based on the total protein in the lane of the test article. Typically samples are resolved using Novex pre-cast gradient 4-20% polyacrylamide gels and stained using Coomassie Blue or silver stain according to manufacturers' recommendations. For Coomassie-blue stained gels, the quantitation of the relative amount of the 31K protein band is assessed using a Molecular Dynamics scanner set with the automatic baseline method. The ratio of 31K MW band peak height to the protein VI band peak height is determined.

Residual 293 Host Cell Protein – Anti-293 Cell reagents, antibodies and standard, are utilized to perform an ELISA to assess residual amounts quantitatively. Additionally the anti-293 cell protein antibodies are used in a Western Blot assay to assess qualitatively any residual 293 host cell proteins. All reagents are from Cygnus Technologies..